



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> METHOD OF REDUCING IMMUNOGENICITY AND INDUCING IMMUNOLOGIC TOLERANCE		
<b>(57) Abstract</b>  The immunogenicity of transfused or transplanted allogenic tissue is reduced by either directly exposing the tissue to ultraviolet (UV) irradiation prior to administering the tissue to the recipient or by inducing a state of tolerance in the recipient to non-UV or UV-irradiated allogenic tissue by prior exposure to UV-irradiated allogenic tissue.		

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METHOD OF REDUCING IMMUNOGENICITY AND  
INDUCING IMMUNOLOGIC TOLERANCE

Description

The present invention relates to a method of reducing the immunogenicity of allogeneic tissues such as blood transfusions and allografts, and to methods of inducing immunologic tolerance to allogeneic tissues in putative recipients.

Background Art

Patients with a variety of disorders receive intermittent or chronic transfusion support, or require tissue grafting to replace a defective organ. Individuals requiring transfusion support have either a genetic or acquired deficiency of one or more blood components that require replacement therapy. Many different products prepared from blood are available for transfusion, including both cellular and plasma components. However, repeated exposure to blood products often results in recipient recognition of the foreign transfused antigens. Such immune recognition of foreign antigens results in a failure to achieve a benefit from the transfusion, and in some circumstances may even cause a transfusion reaction with adverse consequences to the recipient.

Several approaches have been used to either prevent or delay alloimmunization. The majority of these techniques involve giving immunosuppressive therapy to the transfusion recipient to prevent recognition of the transfused foreign antigens. Such immunosuppressive therapy is often inadequate to suppress the recognition process, resulting in alloimmunization in spite of the treatment. Furthermore, the immunosuppressive therapy may have undesirable side effects, including organ toxicity and

immunosuppression of desirable responses, such as recognition and destruction of pathogenic bacteric.

5       Once an immune response to foreign antigens has occurred, there is little evidence that any immunosuppressive therapy is beneficial. Continued adequate transfusion support is possible only if antigen matching between donor and recipient is achieved. Often a matched donor is not available, or for some transfusion products so little is known about the antigen systems involved in the immune response that laboratory methods are not available to appropriately select a matched donor.

15       An alternative approach to preventing alloimmunization, other than immunosuppressing the recipient, is to reduce the immunogenicity of the transfused product. As all transfused blood products are immunogenic and will eventually induce an immune response in most transfused recipients, any procedure that can prevent or at least delay immunization is beneficial. Selecting only antigen compatible donors beginning with the first transfusion is possible in some circumstances, but for the majority of patients either not enough donors are available to continue this process or a matching procedure does not exist.

25       For organ grafting, because there is persistent exposure to foreign tissue antigens, eventual rejection of the grafted tissue occurs. To prevent graft rejection several approaches have been used: recipient immunosuppression, matching tissue antigens of donor and recipient, reducing the immunogenicity of the grafted tissue, or inducing in the recipient a state of tolerance to the foreign antigens of the graft. Furthermore, depending on the tissue being grafted, different approaches may be required to achieve a successful graft, and combined therapies may be additive in their beneficial effects. For example, in bone marrow transplantation massive doses of chemo-radiotherapy are given to the recipient to destroy

the recipient's autologous marrow and to induce immunosuppression to allow engraftment of the donor marrow. Even better results are obtained if marrow donor and recipient are related and well-matched for the major histocompatibility antigen system (HLA). Although post-marrow grafting immunosuppression is usually performed, it is for only a limited time.

In contrast, for kidney grafting lesser degrees of immunosuppressive therapy are required to avoid unacceptable marrow and gastrointestinal toxicity. Furthermore, continuous post-grafting immunosuppression is required. Often a related kidney donor is not available, and lesser degrees of HLA matching between donor and recipient are more often accepted than for bone marrow transplantation.

Another major difference between these two types of tissue grafting is the effect of prior transfusions on engraftment. For kidney graft recipients, prior transfusions, particularly from the intended kidney donor, are beneficial, apparently by inducing some degree of tolerance to the subsequent kidney graft. However, prior blood transfusions before marrow grafting, especially if the blood has come from the intended marrow donor, markedly increase the risk of graft rejection. Thus, although there are similarities in procedures to enhance organ grafts (immunosuppression and donor-recipient HLA matching), there are clear differences in (1) the amount, type, and duration of immunosuppression required; (2) the acceptance of non-HLA identity between organ donor and recipient; and (3) the effect of prior transfusions on enhancing or impairing a subsequent organ graft. Furthermore, even the best combined therapies are not always successful in ensuring a successful organ graft, and there may be substantial toxicities associated with the therapies being used.

Besides using HLA matching and recipient immunosuppression, efforts to directly reduce immunogenicity of the engrafted tissue or to induce

tolerance in the recipient, other than by prior blood transfusions in kidney recipients, have been limited. In bone marrow transplantation, efforts to purify or enrich the marrow graft for stem cells, and to eliminate T-lymphocytes that may be responsible for graft-vs.-host disease (a post-grafting complication), have often resulted in a transplanted marrow that has failed to engraft. Some investigators have stored or cultured the graft in vitro prior to transplantation (skin grafting) to facilitate engraftment. Most of these latter methods to enhance organ grafting have had limited success.

It would be advantageous to avoid immune recognition by the recipient of incompatible donor antigens and the consequent destruction of allogeneic tissue following transfusion or transplantation.

#### Disclosure of Invention

Briefly stated, the present invention is directed toward (a) methods for reducing the immunogenicity of blood products and transfused or transplanted tissue; and (b) methods and compositions for substantially reducing sensitization of a recipient to subsequently introduced blood products, transfused or transplanted tissue. Compositions of blood products or transfused or transplanted tissue for use as active therapeutic substances are also disclosed.

In one aspect of the present invention, a method is disclosed for reducing the immunogenicity of blood products. Suitable blood products include red cells, white cells, platelets, and plasma constituents. Plasma constituents include, for example, albumin, clotting factor proteins, gamma globulin and antithrombin-III. The method generally involves exposing the blood products to a source of UV irradiation sufficient to reduce the immunogenicity of the blood products. Generally, the UV irradiation results in an exposure of from about 12 J/m<sup>2</sup> to about 1350 J/m<sup>2</sup>.

In one embodiment of this method, the dose of UV irradiation does not substantially interfere with the viability or physiological function of the blood products. The threshold exposure to UV irradiation, at which substantial interference with viability or physiological function occurs, can be determined by reference to identifiable parameters of viability and function for unmanipulated, non-UV irradiated tissue using standard laboratory testing protocols. Where the blood product is platelets, for instance, the UV irradiation results in an exposure of from about 12 J/m<sup>2</sup> to about 36 J/m<sup>2</sup>. Where the blood product is red blood cells, one specific embodiment comprises UV irradiation resulting in an exposure of approximately 1350 J/m<sup>2</sup>.

A second aspect of the present invention is directed toward a method for reducing the immunogenicity of transfused or transplanted tissue in vitro. Suitable transfused or transplanted tissues include tissues such as bone marrow, skin, bone, and cornea, and organs including heart, lung, liver, pancreas and kidney. The method generally involves exposing the transfused or transplanted tissue to a source of UV irradiation sufficient to reduce the immunogenicity of the transfused or transplanted tissue.

Within this method, generally the UV irradiation results in an exposure of from about 12 J/m<sup>2</sup> to about 1350 J/m<sup>2</sup>. In one embodiment of this method, the dose of UV irradiation does not substantially interfere with the viability or physiological function of the transfused or transplanted tissue. As noted above, the threshold exposure to the UV irradiation at which substantial interference with viability or physiological function occurs can be determined by using standard laboratory testing protocols. Where the transfused or transplanted tissue is pancreatic cells, for instance, the UV irradiation results in an exposure that should not exceed 900 J/m<sup>2</sup>.

A third aspect of the present invention discloses a series of compositions useful as active therapeutic substances. These compositions include blood products, or transfused or transplanted tissue exposed to a source of UV irradiation sufficient to reduce the immunogenicity of the blood products, transfused or transplanted tissue. Another group of compositions includes blood products and transfused or transplanted tissue exposed to a source of UV irradiation sufficient to substantially reduce the sensitization of a recipient to subsequently introduced blood products, transfused or transplanted tissue. As described below, these compositions have a variety of uses as active therapeutic substances.

Other aspects of the present invention will become evident upon reference to the following detailed description.

#### Best Mode for Carrying Out the Invention

The transfusion of whole blood or blood products may be given to patients who are deficient in one or more substances that are present in whole blood. Patients requiring transfusions may have either a congenital or acquired deficiency of the substance being replaced by transfusion. The patient's deficiency may occur for a limited period of time or may be chronic. For those individuals with a chronic deficiency of a component of whole blood, continued beneficial effects of the transfusion are dependent upon the recipient not developing an immune response to the transfused material. For some transfused substances, the antigenic nature of the product is well-defined and long-term compatibility between donor and recipient is often achieved. For example, the major antigens on the surface of red cells that need to be matched between donor and recipient are the A and B antigens and the RhD antigens. The gene frequency of these antigens in the population makes it relatively easy to ensure finding a compatible donor for almost all recipients.



In contrast, platelets express HLA antigens on their surface as the major alloantigenic system that must be matched between donor and recipient to ensure compatibility. However, the genetic heterogeneity of the HLA system makes matching of this complex antigen system between donor and recipient difficult. Thus, patients requiring chronic platelet transfusion support often become alloimmunized to HLA antigens that are different between donor and recipient. In addition, there are platelet-specific antigens which also may need to be matched between donor and recipient. Thus, the development of alloantibodies to donor platelets often occurs and is difficult to manage.

Similarly, organ and tissue transplants generally require antigenic compatibility between donor and recipient for the HLA antigen system as well as the ABO system. Furthermore, in spite of compatibility for all the recognized blood group and histocompatibility antigen systems, continued functioning of the transplanted organ or tissue requires that temporary or permanent immunosuppressive therapy be provided to the recipient. Often, either the degree of antigenic compatibility between donor and recipient and/or the effectiveness of the immunosuppressive therapy given to the recipient is not sufficient to prevent graft rejection. Because the transplanted organ or tissue is often vital to the well-being of the recipient, failure of engraftment may result in substantial recipient morbidity or mortality.

Thus, any procedure which can reduce the immunogenicity of the transfused or transplanted tissue or, alternatively, reduce sensitization to the subsequent introduction of these tissues, may result in a substantial benefit to the recipient. One of the purposes of this invention is to provide a method of reducing immunogenicity or reducing sensitization, so that individuals who need replacement of either transfused or transplanted tissue can have a successful outcome. In addition, it is believed that the techniques used to reduce immunogenicity and

reduce sensitization are sufficiently efficacious that the requirement for antigenic compatibility between donor and recipient, in order to ensure a successful transfusion or transplantation procedure, is obviated.

5 Pursuant to this invention, the immunogenicity of the transfused or transplanted tissue is reduced by directly exposing the tissue to ultraviolet (UV) irradiation prior to administering the tissue to the recipient. Alternatively, a state of tolerance in the  
10 recipient to non-UV or UV-exposed allogeneic tissue or organs may be induced by prior exposure to UV-irradiated allogeneic tissue.

In the first situation, where the objective is to reduce the immunogenicity of the transfused or transplanted  
15 tissue or organ, it is important to determine that the UV-exposure does not substantially interfere with the viability or physiologic function of the allogeneic tissue. The threshold exposure of UV-irradiation, at which substantial interference with viability or physiologic  
20 function occurs, can be determined by reference to tissue-specific parameters of viability and function for unmanipulated, non-UV-irradiated tissue or organs, using standard laboratory testing protocols. (Lindahl-Kissling, K. and J. Safwenberg, Int. Arch. Allergy 41: 670, 1971; Dutcher et al., Blood 58: 1007, 1981; Weiden et al., J. Immunol. 117: 143, 1976; Granstein et al., J. Immunol. 132: 2210, 1984).

For some transplanted tissues, a UV dose effective in reducing immunogenicity may be impossible to  
30 administer due to the bulk nature of the engrafting tissue, e.g., cardiac and kidney grafts, recognizing the low penetrance of UV-irradiation. Alternatively, for some tissues, the threshold exposure at which biologic activity is substantially unimpaired may be too low to prevent  
35 immune recognition when the tissue is administered to the recipient. Many experimental and clinical studies have attempted to change the immunoresponse of the prospective

transplant recipient by transfusing normal blood products prior to transplantation. Although this approach has proven beneficial in many instances (i.e., graft survival was improved), it was detrimental in others because the recipient became sensitized and the subsequent grafts were rejected in an accelerated fashion. UV-exposed blood may have the same beneficial effect as normal blood and, in addition, may not lead to sensitization. The term sensitization, as used in the present invention, refers to the presence of antibodies or lymphocytes in the recipient that are either directed against or capable of recognizing donor material. This recognition will normally result in destruction of the transfused product or rejection of the graft. For these circumstances, a UV-irradiated tolerizing tissue can be administered to the recipient prior to or concurrent with the administration of the transfused or transplanted tissue, such as an organ.

For the purpose of reducing sensitization or inducing tolerance, the dose of UV-irradiation need not necessarily maintain the biologic activity of the UV-irradiated blood product or tissue being used to reduce sensitization or induce tolerance. There may be no requirement for the tolerizing tissue or blood product to be derived from the same individual as the one from whom the subsequent transfusion or graft is obtained, nor is there even a requirement that they be derived from a related or histologically identical individual. In fact, as outlined in Table 1, the donor of the UV-exposed blood product or tissue may be either related or unrelated to the recipient of the transfused or transplanted tissue or blood product and, furthermore, may not be required to be antigenically matched with the recipient of the transfused and/or transplanted tissue or blood product.

TABLE 1

	<u>Donor</u>	<u>Recipient</u>
5	UV-Exposed Blood Products or Tissue	UV-Exposed or Normal Transfused/Transplanted Tissue or Blood Products
	<u>Blood Products</u>	<u>Organs</u> (e.g., heart, lung, liver, pancreas, kidney, etc.) <u>Tissue</u> (e.g., bone marrow, skin, cornea, bone, etc.)
10	Whole blood, red blood cells, white blood cells, platelets and plasma con- stituents (albumin, clotting factors, gamma globulin, antithrombin-III, etc.)	<u>Blood Products</u> Whole blood, red blood cells, white blood cells, platelets and plasma constituents (albumin, clotting factors, gamma globulin, antithrombin- III, etc.)
15	<u>Tissue</u> (e.g., bone marrow, skin, cornea, bone, etc.,	
20		

	Relationship Between Donor of UV-Exposed Blood Product and
25	<u>Transfused/Transplanted Recipient</u>
	Autologous or Syngeneic
	Related -- Antigenically matched, or mismatched
	Unrelated -- Antigenically matched, or mismatched

30 To summarize the principles of this invention,  
three illustrative examples are provided. Transfusions of  
35 normal blood from the intended donor prior to bone marrow  
transplantation will result in sensitization of the  
recipient in all instances, as determined by marrow graft  
rejection. If, however, blood products are UV irradiated  
prior to transfusion, sensitization can be substantially,  
if not entirely, eliminated, as shown in Example 1, by

successful bone marrow engraftment. Thus, recipients given UV irradiated blood, in contrast to recipients given normal blood products, behave similarly to recipients who have not been given a transfusion. In the illustration, the donor of the blood and bone marrow was a DLA-matched littermate of the transfused and transplanted recipient.

In the second example, the process of reducing the immunogenicity of a transfused blood component (platelets), while maintaining viability and function of the UV-exposed material to allow for effective platelet support, is detailed. The effectiveness of the procedure in reducing the immunogenicity of the transfused material is illustrated by the fact that the procedure was successful in preventing alloimmune platelet destruction between randomly selected unrelated donor and recipient pairs.

In addition, the latter studies provide evidence that in at least a portion of the recipients, a state of unresponsiveness (non-specific tolerance) was induced by the transfusion of UV-irradiated blood products, since subsequent transfusion of normal blood products did not result in sensitization.

The following examples are offered by way of illustration, and not by way of limitation.

#### EXAMPLE 1

In these experiments using a canine model, it is demonstrated that exposure of whole blood to ultraviolet light before transfusion prevents immunization and allows for subsequent marrow engraftment.

Normal dogs given 9.2 Gy of total body irradiation and bone marrow transplants from DLA (major histocompatibility complex) - identical littermate donors generally achieve sustained engraftment and become long-term survivors. However, if recipient dogs are transfused with whole blood from the marrow donor before transplantation, the marrow graft is always rejected and

the recipient dogs uniformly die with marrow aplasia. Such graft rejections are apparently the result of transfusion-induced sensitization of the recipient against minor (non-DLA) histocompatibility antigens of the donor.

5           In this Example, normal dogs were given 9.2 Gy total body irradiation, hemopoietic marrow infusion from DLA-identical littermates, and post-transplant conditioning as described in Blood 54:477, 1979. Prior to marrow grafting, recipients were given 3 transfusions, on days 24,  
10 17, and 10 before transplantation. The transfusions consisted of either unmanipulated, UV-irradiated or sham-irradiated whole blood from the marrow donor. The following procedure was used. Whole blood (50 ml/transfusion) was obtained from a donor Beagle dog by  
15 venipuncture with syringes containing preservative-free heparin to prevent coagulation. The blood was then diluted 1:1.5 with Waymouth's Minimal Medium. Aliquots of 7.5 ml were placed in 10 plastic dishes (Falcon #3003), resulting in a layer of 1.5 mm thickness in each dish. The uncovered  
20 plates, placed on a shaker platform to assure continual mixing of the blood, were then exposed for 30 minutes to UV irradiation (220-300 nm wavelength) from a germicidal lamp (General Electric) at an intensity of 750 uW/cm<sup>2</sup> (total exposure 1.35 mJ/cm<sup>2</sup>), as determined with a Black Ray  
25 shortwave UV meter (U.V. Products). Cells were then quantitatively recovered from the dishes, collected into syringes and injected into a recipient dog. Sham-irradiated blood was handled in an identical fashion, except that it was exposed to visible light instead of UV  
30 light.

Table 2 shows the results of these experiments.

TABLE 2

5	Group	Trans- fusions	No. of Dogs			Incidence Sustained Graft
			Studied	With	With	
				Graft Rejection	Sustained Graft	
10	6	None	60	1	59	98%
	7	Whole blood unmanipulated	21	21	0	0%
15	8	Whole blood, UV-irradiated	10	0	10	100%
20	9	Whole blood, sham- irradiated	4	4	0	0%

The majority of the dogs in Groups 6 and 7 were included in the above-cited previous report. Of the ten dogs in Group 8 that had a sustained graft, three died with septicemia at 8, 9 and 13 days post-grafting, respectively. Engraftment was documented by rising granulocyte counts following the post-irradiation nadir, and by the presence of erythroid and myeloid precursor cells (total cellularity 10-25% of normal) on marrow samples obtained at autopsy. The other seven dogs are surviving after more than 100 days; in these dogs sustained engraftment was documented by the presence of the donor sex karyotype in all metaphase spreads from bone marrow and peripheral blood (four dogs) or by conversion to the donor erythrocyte antigen pattern (three dogs). Dogs in group 9 died on days 10, 11, 11, and 13, respectively, with septicemia. Marrow cellularity at autopsy was less than 5% in all of these dogs. Cells were

composed of plasma cells and reticulum cells; hemopoietic precursor cells were absent.

5 All of the dogs that were given transfusions of UV-exposed blood prior to marrow transplantation sustained engraftment and behaved like dogs not given any transfusions before transplantation. In contrast, all of the control dogs, transfused before transplantation with blood exposed to light in the visible range, rather than the UV range, failed to achieve substantial engraftment and died with bone marrow aplasia, similar to the dogs given transfusions of unmanipulated whole blood. Thus, exposure of blood to UV light appears to abrogate the sensitizing ability of blood, and allows for subsequent successful engraftment.

15 In patients undergoing marrow transplantation for various diseases, especially severe aplastic anemia, exposure to transfusion products before marrow transplantation, as in our dog model, leads to a significant risk of subsequent marrow graft rejection. While patients who are untransfused at the time of marrow transplantation usually achieve sustained engraftment and become healthy survivors, patients who have been given transfusions before transplantation have been reported in previous studies to have an incidence of graft rejection as high as 60%. Since graft rejection is usually not compatible with the patient's survival, it is highly desirable to prevent sensitization of the patient before transplantation. The method described herein provides such an approach. Furthermore, it is contemplated that this method may result in tolerance of other transplants, e.g., kidney, heart, etc., as well.

#### EXAMPLE 2

35 Platelet transfusion experiments were performed between unrelated donor-recipient dog pairs that were randomly selected from a pool of animals. The experimental design involved preparing a platelet concentrate from 50 ml



of blood drawn from a donor animal, and radiolabelling the donor's platelets with  $^{51}\text{Cr}$  as described by Weiden et al., J. Immunol. 117:143-150, 1976. After transfusion of the radiolabelled donor dog's platelets into a recipient, serial blood samples were drawn from the recipient to determine the disappearance rate of the donor's platelets from the recipient's circulation. If only 5% or less of the radiolabelled donor platelets were circulating in the recipient at 24 hours post-transfusion, the animal was considered to be alloimmunized to the donor's platelets.

In 21 recipients, weekly transfusion of a single, unrelated donor's platelets resulted in immunization in 20 recipients (95%) after an average of  $3.1 \pm 0.7$  transfusions (range 2-12). Of these non-irradiated control transfusion recipients, 18/21 (86%) were immunized by  $\leq 8$  donor transfusions.

In an effort to prevent immune recognition of the transfused platelets, 12 randomly-selected, unrelated donor-recipient pairs were transfused with UV-irradiated platelets. Before these transfusion studies were begun, 14 animals had their autologous platelets UV-irradiated, and after radiolabelling these platelets were returned to the donor. These experiments showed that UV-irradiation doses of greater than 600 uwatts/cm<sup>2</sup> for more than 10 minutes (total dose of 360 J/m<sup>2</sup>) resulted in loss of platelet viability, as documented by an absent or reduced autologous platelet survival determination. Based on these autologous studies, for the experimental paired donor-recipient transfusion studies doses of UV irradiation that did not produce loss of platelet viability were selected and used.

Specifically, 12 recipient animals received  $^{51}\text{Cr}$  radiolabelled donor platelets that had been exposed to 200, 400, or 600 uwatts of UV irradiation/cm<sup>2</sup> for one minute, delivered by a germocidal lamp (220-300 nm wavelength) (total dose of 12, 24, and 36 J/m<sup>2</sup>, respectively). The platelets were exposed to the UV-irradiation by placing them at a depth of about 1-2 cm in an open Petri dish which

was continually agitated through out the UV exposure. The same randomly-selected donor-recipient pairs were used for a maximum of eight weekly transfusions or until alloimmunization occurred. Only 1/12 (8%) of the recipient dogs who received UV-irradiated donor platelets became immunized.

Next, in the 11 non-immunized recipients (after the eight weeks of UV-irradiated, unrelated donor platelets had been given), an additional 8 weeks of non-UV-irradiated platelet transfusions from the same donor were given. Only 3/11 (27%) of these recipients were able to recognize the non-UV-irradiated platelets from their unrelated donor. In addition, if non-UV-irradiated platelets from a new unrelated donor were subsequently given to the 8 recipients who had failed to recognize non-UV-irradiated platelets from their original donor, 10/23 (43%) of these donors failed to cause alloimmunization in the recipient even after 8 transfusions. The other 3 were immunized by the new donor's platelets by 1, 1 and 9 transfusions, respectively. These data demonstrate that tolerance to non-UV-irradiated transfused donor platelets was induced in these recipients by prior transfusions of UV-irradiated platelets, and that the tolerance was not specific for the original donor's platelets.

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## EXAMPLE 3

UV exposure as described in Example 1 is compatible with useful red cell transfusion support. In one dog the survival of UV-exposed  $^{51}\text{Cr}$  labelled red blood cells was measured according to standard techniques. The  $T_{1/2}$  was 17 days, which compares to a range of 17-24 days measured in dogs given unmanipulated transfusions.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not to be limited except as by the appended claims.

Claims

1. A method of reducing the immunogenicity of blood products in vitro, comprising:  
    exposing the blood products to a source of UV irradiation sufficient to reduce the immunogenicity of the blood products.
2. The method of claim 1 wherein the blood product is selected from the group consisting of red cells, white cells, platelets and plasma constituents.
3. The method of claims 1 or 2 wherein the UV irradiation results in an exposure of from about 12 J/m<sup>2</sup> to about 1350 J/m<sup>2</sup>.
4. The method of claim 1 wherein the dose of UV irradiation does not substantially interfere with the viability or physiological function of the blood products.
5. The method of claim 4 wherein the blood product is platelets and the UV irradiation results in an exposure of from about 12 J/m<sup>2</sup> to about 36 J/m<sup>2</sup>.
6. The method of claim 4 wherein the blood product is red blood cells and the UV irradiation results in an exposure of approximately 1350 J/m<sup>2</sup>.
7. A method of reducing the immunogenicity of transfused or transplanted tissue in vitro comprising:  
    exposing the transfused or transplanted tissue to a source of UV irradiation sufficient to reduce the immunogenicity of the transfused or transplanted tissue.
8. The method of claim 7 wherein the transfused or transplanted tissue is selected from the group consisting of

bone marrow, skin pancreas, bone, liver, heart/lung, kidney and cornea.

9. The method of claims 7 or 8 wherein the UV irradiation results in an exposure of from about 12 J/m<sup>2</sup> to about 1350 J/m<sup>2</sup>.

10. The method of claim 7 wherein the dose of UV irradiation does not substantially interfere with the viability or physiological function of the transfused or transplanted tissue.

11. The method of claim 10 wherein the transfused or transplanted tissue is pancreatic cells and the UV irradiation results in an exposure that does not exceed 900 J/m<sup>2</sup>.

12. A composition comprising transfused or transplanted tissue exposed to a source of UV irradiation sufficient to reduce the immunogenicity of the transfused or transplanted tissue for use as an active therapeutic substance.

13. A composition comprising blood products exposed to a source of UV irradiation sufficient to reduce the immunogenicity of the blood products for use as an active therapeutic substance.


14. A composition comprising transfused or transplanted tissue exposed to a source of UV irradiation sufficient to substantially reduce sensitization of a recipient to subsequently introduced blood products, transfused or transplanted tissue, for use as an active therapeutic substance.

15. A composition comprising blood products exposed to a source of UV irradiation sufficient to substantially reduce sensitization of a recipient to subsequently introduced

blood products, transfused or transplanted tissue, for use as an active therapeutic substance.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/01409

<b>I. CLASSIFICATION F SUBJECT MATTER</b> (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>4</sup> : A 61 K 41/00; A 61 M 1/36		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
IPC <sup>4</sup>	A 61 L; A 61 K; A 61 M; G 21 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched *		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT *</b>		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	WO, A, 85/00954 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 14 March 1985 see claims 8-21; page 1, lines 10-26; page 3, lines 24-31; pages 4-6, line 10; page 13; page 14, lines 1-14	1-15
X	US, A, 3973001 (K.H. JAEGER et al.) 3 August 1976 see column 1, lines 14-21	1,2,12-15
P,X	US, A, 4576143 (W.T. CLARK, III) 18 March 1986 see the abstract; claim 1; column 6, lines 61-64	1,2,7,12-15
X	FR, A, 975851 (M. DELAVILLE) 12 March 1951 see column 1, lines 1-4	1,2,7,12-15
A	US, A, 3683183 (T.A. VIZZINU et al.) 8 August 1972 see column 1, lines 10-20	1,2,7
A	US, A, 4456589 (D.G. HOLMAN et al.) 26 June 1984	
A	EP, A, 0138489 (EXTRA CORPOREAL MEDICAL SPECIALITIES INC.) 24 April 1985	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
9th October 1986	13 NOV 1986	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	M. VAN MOL 	

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO.

PCT/US 86/01409 (SA 13818)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 16/10/86

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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US-A- 3973001	03/08/76	None	
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For more details about this annex :  
see Official Journal of the European Patent Office, No. 12/82